

Contributions of Genomics to NOAA's
Coastal Ocean Science

Understanding the Causes and Impacts of Harmful Algal Blooms



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**Hollings
Marine
Laboratory**



**Center for Coastal
Environmental Health and
Biomolecular Research**



NOAA/NOS/NCCOS/CCEHBR

**College of Charleston
Grice Marine Lab**



**South Carolina
Marine Resources
Research Institute**



**Medical University
of South Carolina**







(NOAA, NIST, DNR,
CofC, MUSC)

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Hollings Marine Laboratory



Developing advanced technology for linking marine ecosystem condition and public health & well-being

- NCCOS Center for Human Health Risk at HML
- NOAA Center of Excellence for Oceans and Human Health at the HML
- SC Center of Economic Excellence in Marine Genomics

Some definitions

Genome is the sum total of all genes in an organism

Genomics is the simultaneous study of all genes in the genome (whereas genetics studies one gene at a time)

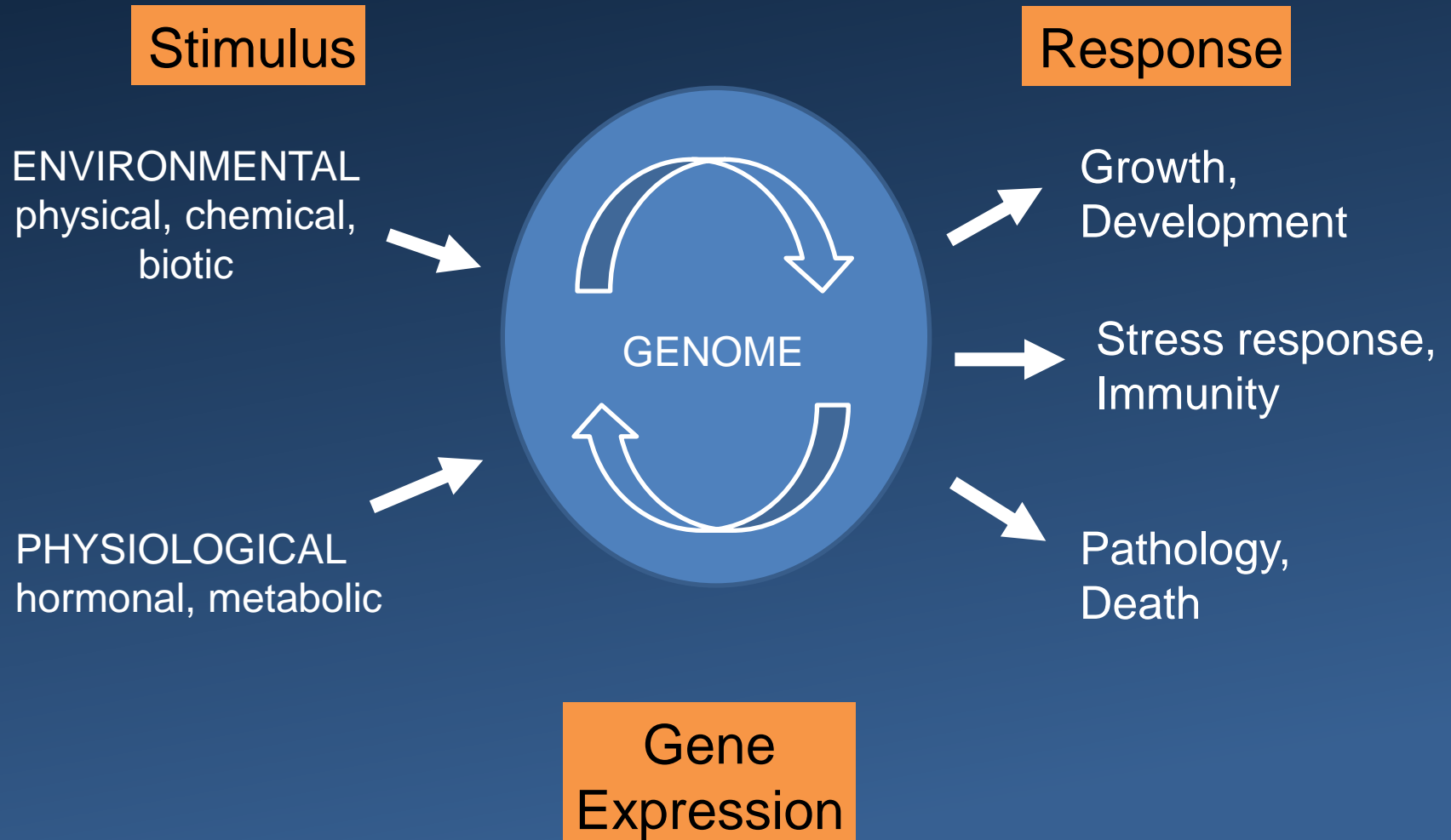
Structural genomics – sequence and gene structure

Functional genomics – response of the genome to change

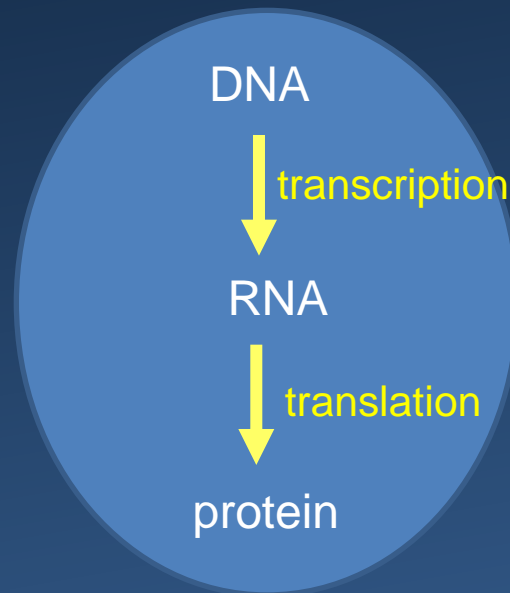
Metagenomics – identification of all organisms in a sample based on gene sequences and (**metatranscriptomics**) what they are doing

Toxicogenomics – identification of genes/metabolic pathways responding to toxin exposure. Provides predictive power.

Functional Genomics Paradigm



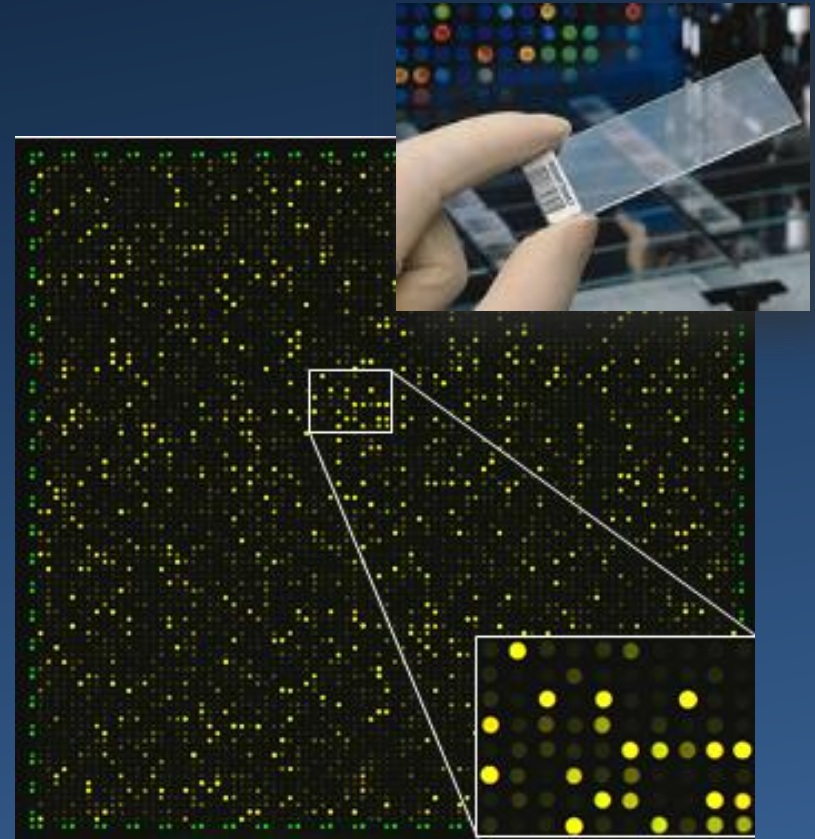
Gene Expression



→ In Functional Genomics
RNA expression is a
proxy for gene activity

Microarrays: Workhorse Tools for Functional Genomics

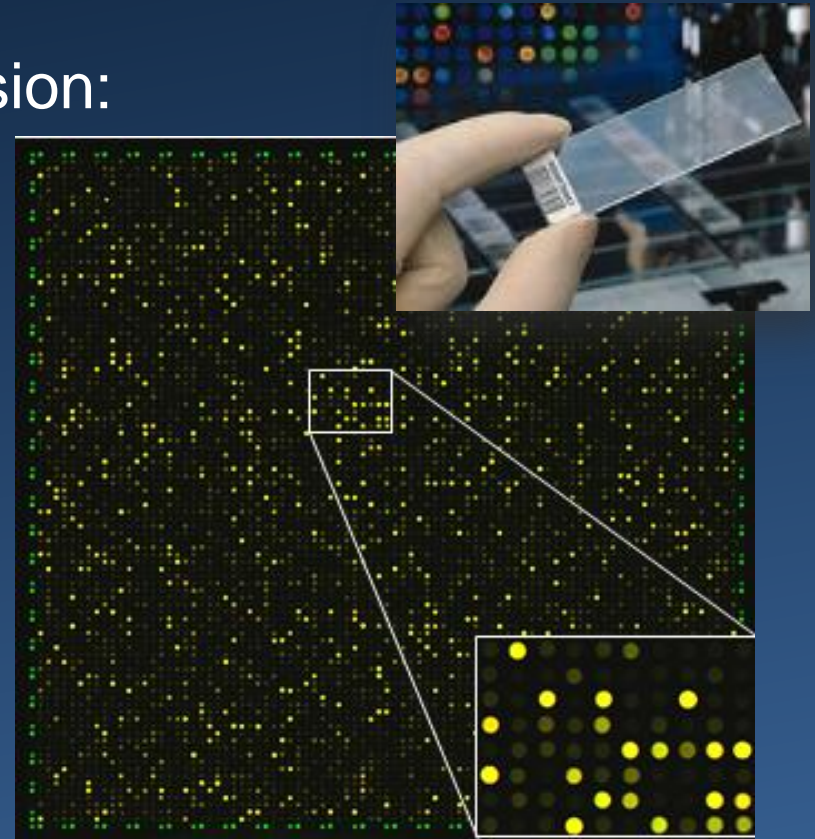
- Glass slide containing probes (short sequences) representing each unique gene in an organism
- Each spot (probe) is 5 nm in diameter
- Most arrays contain 15K – 44K probes (can represent the whole genome)



Microarrays: Workhorse Tools for Functional Genomics

To measure global gene expression:

1. Extract total RNA from an organism or tissue
2. Label total RNA with a fluorescent dye
3. Incubate the labeled RNA sample with the array.
4. Each individual RNA will bind only to its cognate gene probe.
5. Fluorescence intensity reflects how highly that gene is expressed



Microarray data



Identifies significantly regulated genes (up or down):



Gene Networks,
Pathways



Hypothesis about
mechanisms



Classifiers



Prediction
models

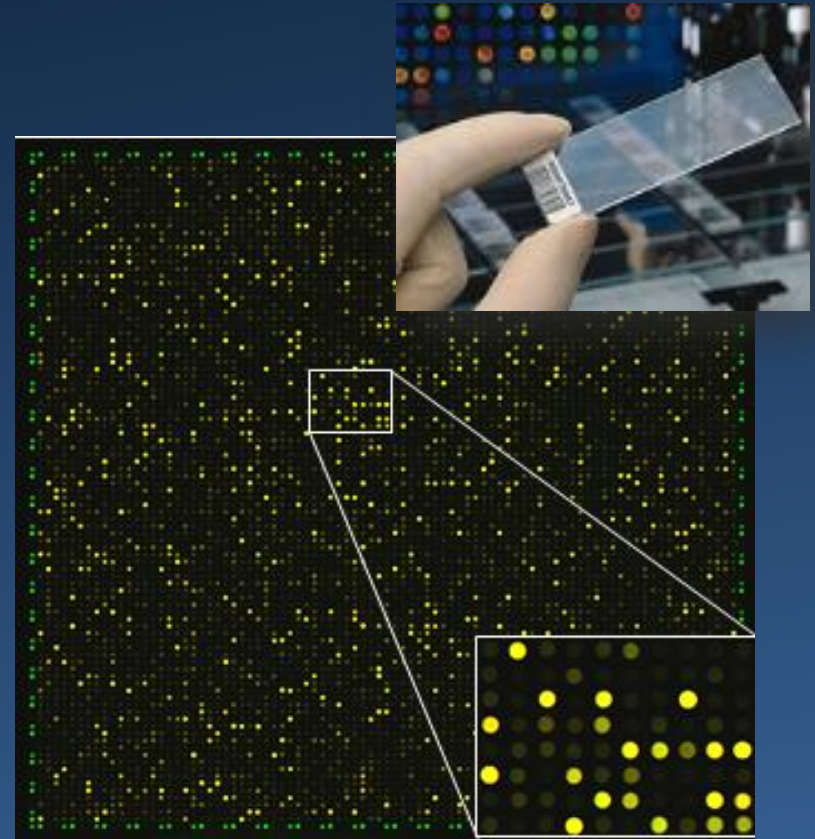
Microarrays: Workhorse Tools for Functional Genomics

Microarrays developed at HML/CCEHBR
for non-model marine species:

eastern oyster
white shrimp
striped bass
Fundulus
grass shrimp
mussel
amphipod
bottlenose dolphin
California sea lion
Karenia brevis – FL red tide

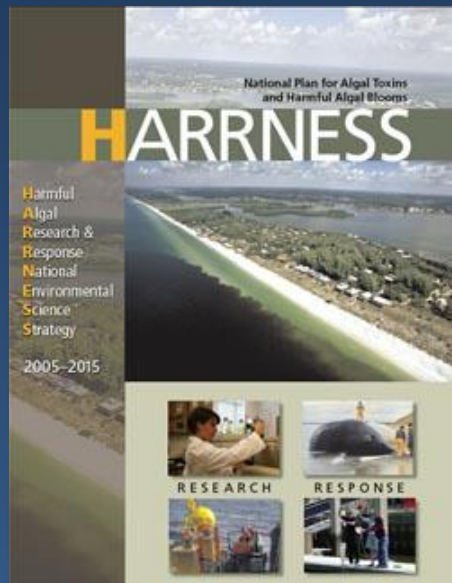
Microarrays employed for model
species :

mouse
human



HAB Functional Genomics: Identifying Molecular Processes Regulating Bloom Growth and Toxicity

Harmful Algal Bloom Research and Response:
A National Environmental Science Strategy 2005-2015



Models and Forecasting

- Develop and improve species-specific models that link physical-biological models
- Develop genome sequences for selected HABs to guide physiological and behavioral studies

Control and Mitigation

- Identify genes and biosynthetic pathways linked to toxin production
- Develop effective, environmentally sound techniques to control HABs and their impacts
- Strengthen understanding of life histories, ecophysiology, behavior, and in situ growth and death rates

Karenia brevis

Responsible for “red tides” in the Gulf of Mexico

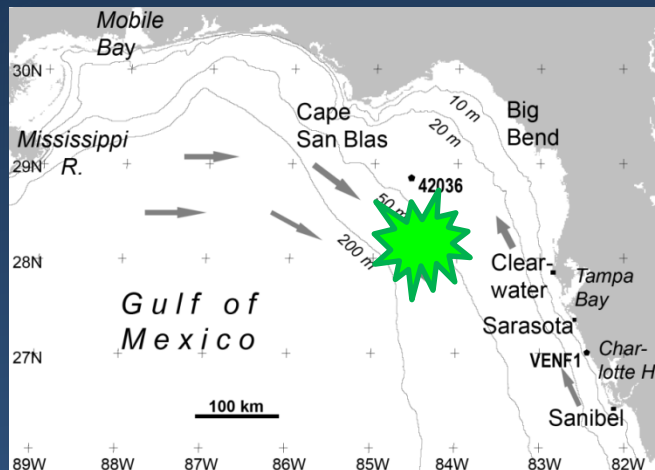
Often dominates thousands of square km of ocean and may persist for many months



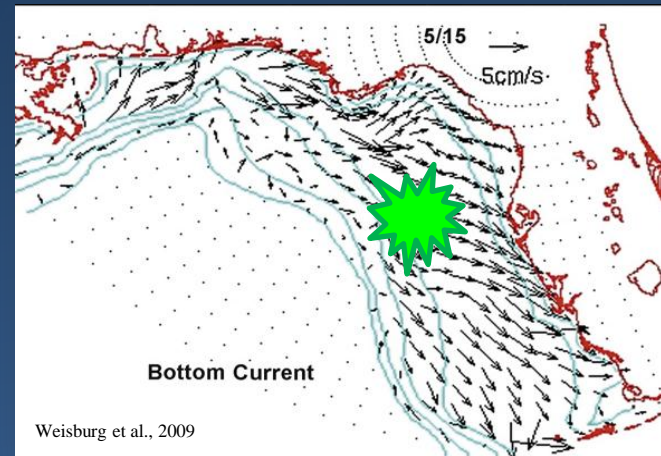


How do blooms form? The *K. brevis* conundrum:

- slow growing
- adapted to low nutrient conditions
- adapted to low light
- blooms initiate offshore

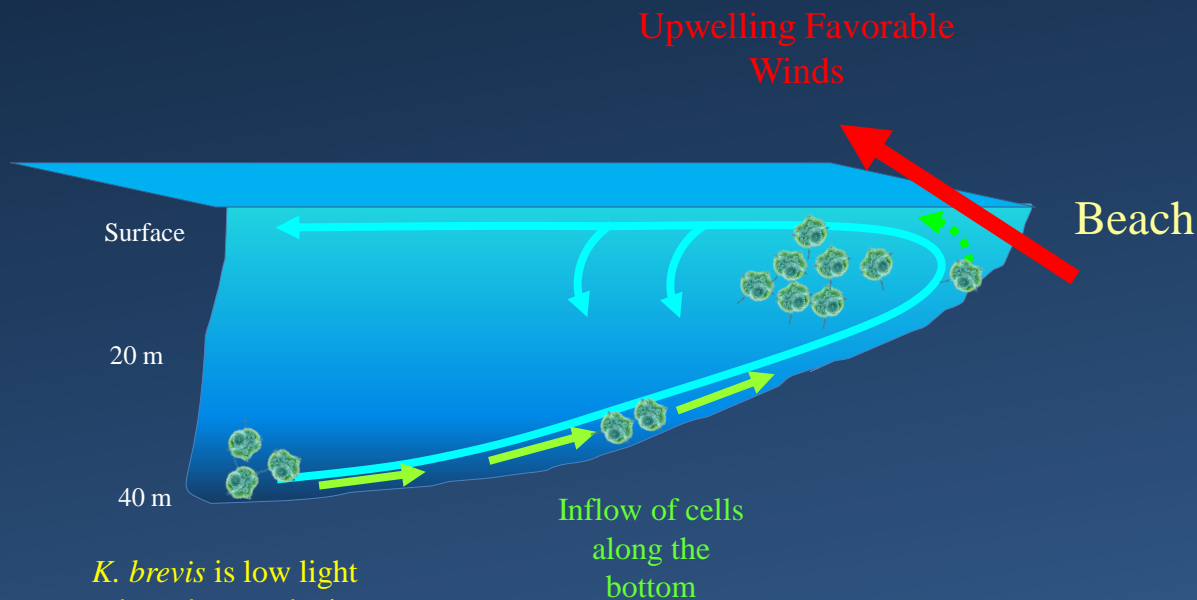


Surface currents



Bottom currents

Shoreward bottom transport & concentration of *K. brevis* by upwelling

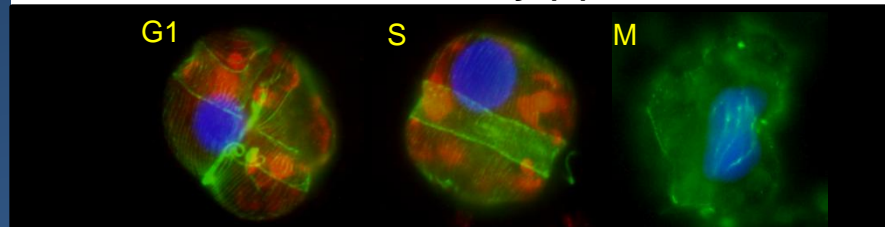
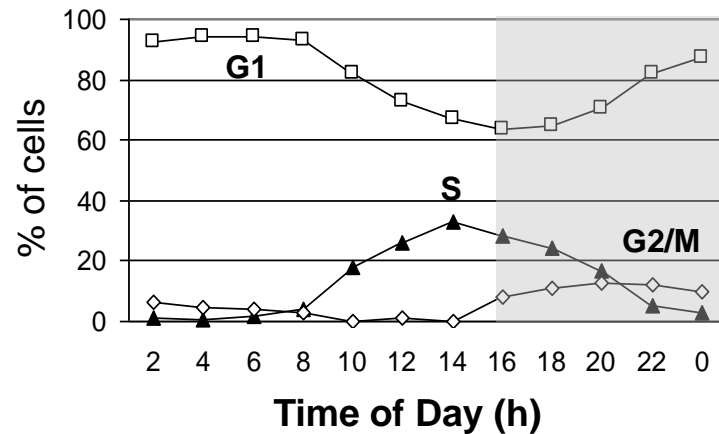
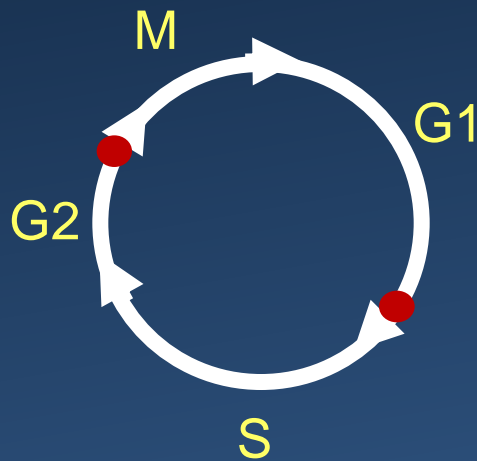


K. brevis is low light adapted; can obtain nutrients in bottom water and sediments

Key Processes Regulating Bloom Growth and Toxicity:

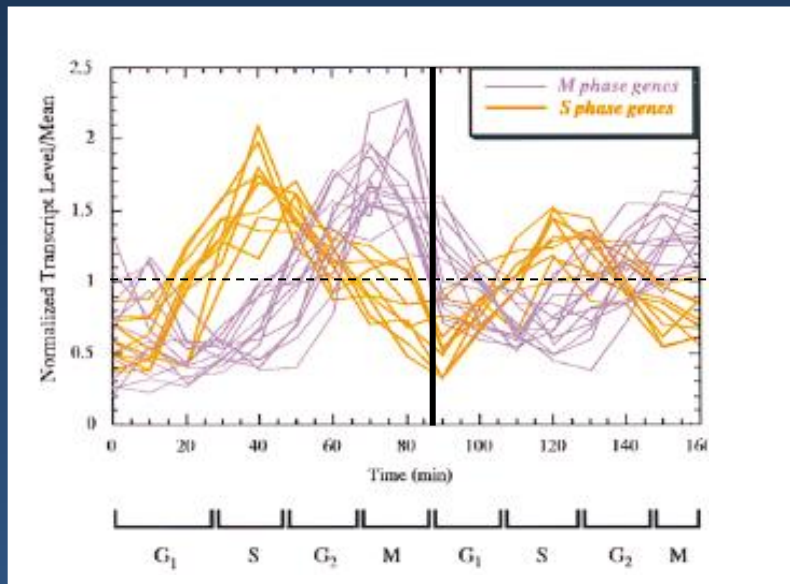
- ✓ • cell division
- toxin biosynthesis
- ✓ • nutrient utilization
- stress responses
- cell death

Cell Division in *K. brevis* is under Diurnal Control



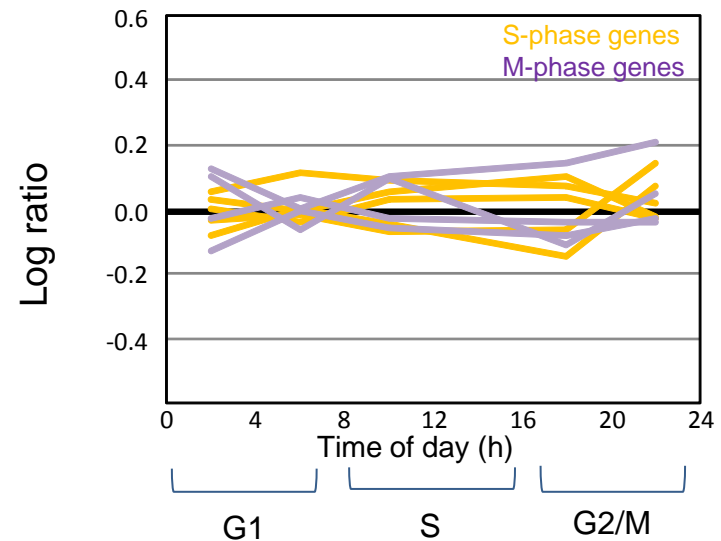
Microarray Studies: expression of genes specific for DNA synthesis (S-phase) and division (M-phase) can be used as markers for actively growing cells

Yeast



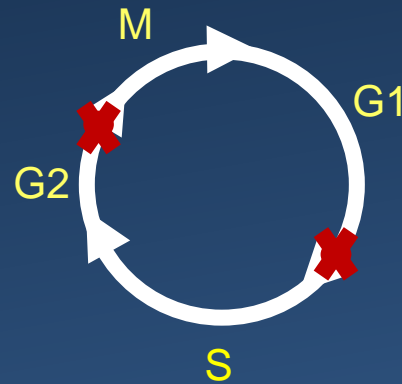
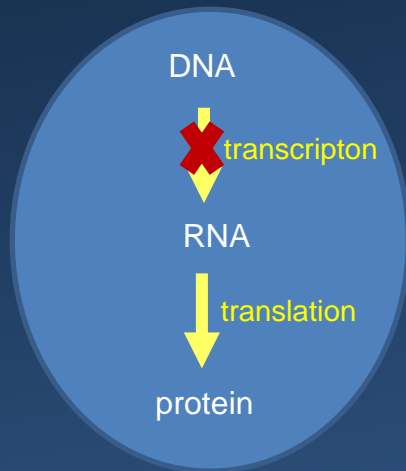
Cho et al 1998

Karenia

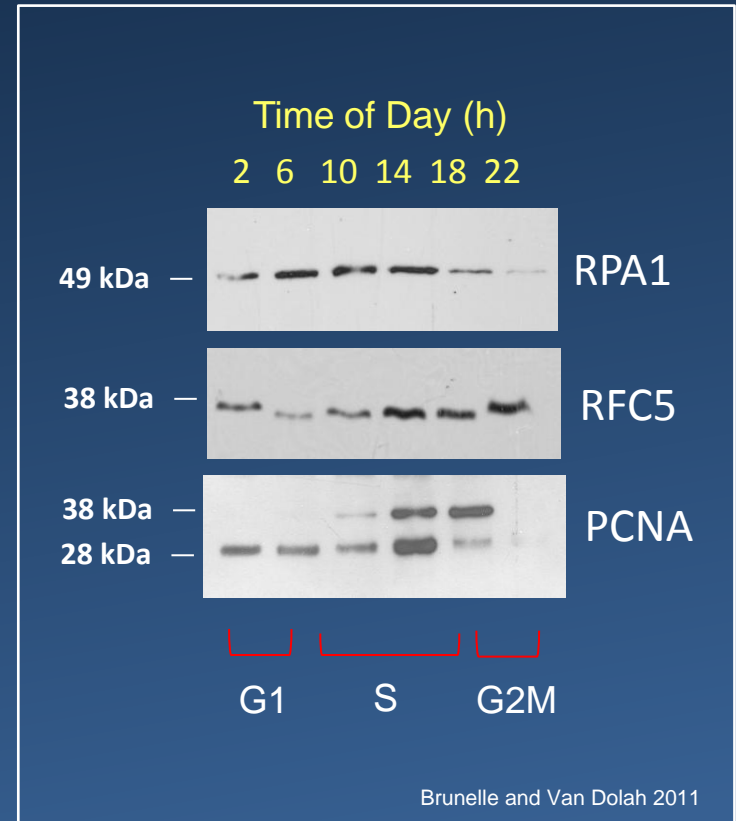


Van Dolah et al 2007

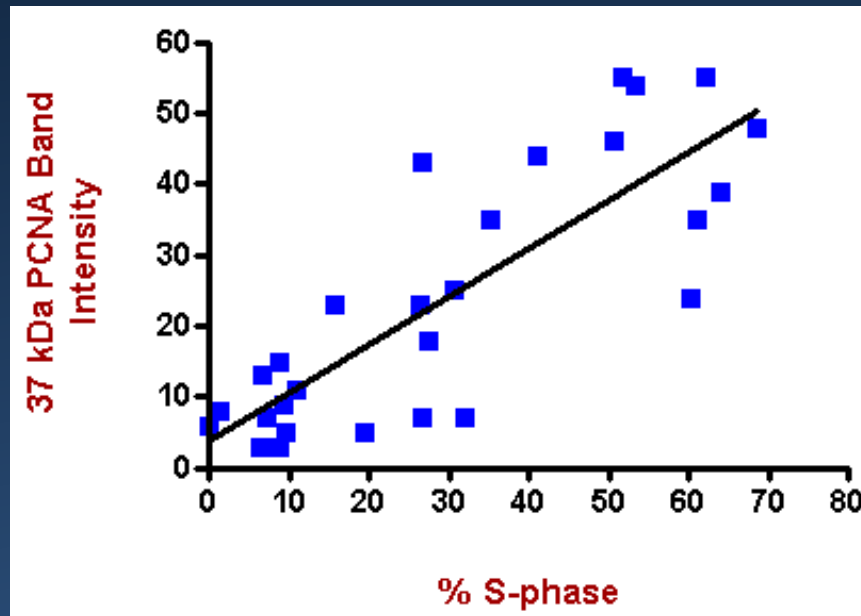
Cell Cycle Regulation in *K. brevis* Occurs at the Protein Level



Expression of S-phase proteins



Cell Cycle Proteins as Potential Indicators of Bloom Growth Status



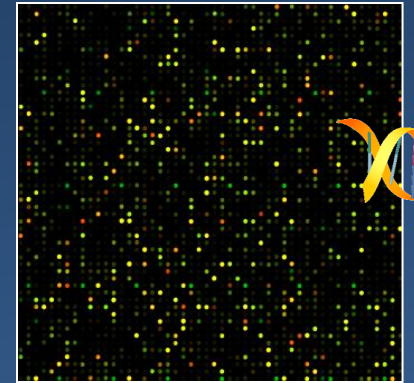
- Significant relationship between cell cycle protein expression and % of dividing cells



- Blooms of *K. brevis* often persist at high density in coastal waters
- What sources of nitrogen and phosphorus support dense blooms of *K. brevis* in coastal waters?

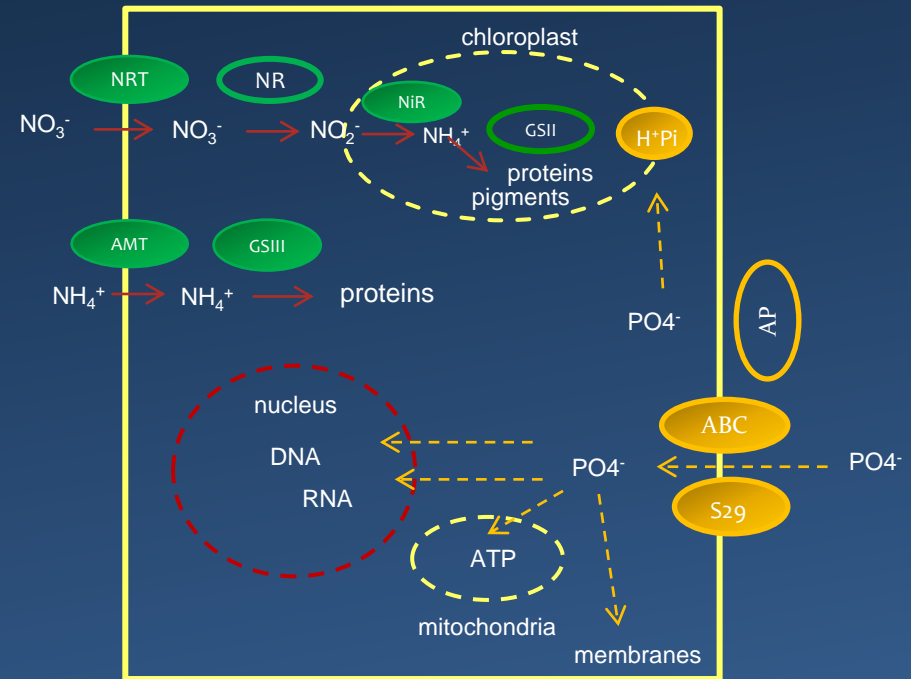
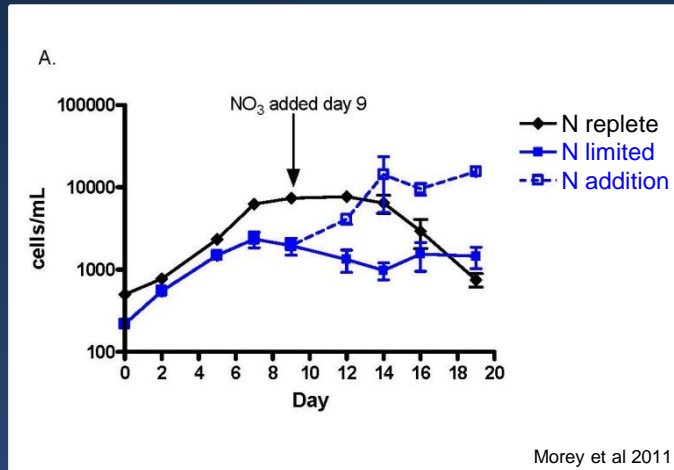


- In diatoms expression of N assimilation genes can serve as biomarkers for N status and source
- In cyanobacteria N- and P- responsive gene sets have been identified using microarrays



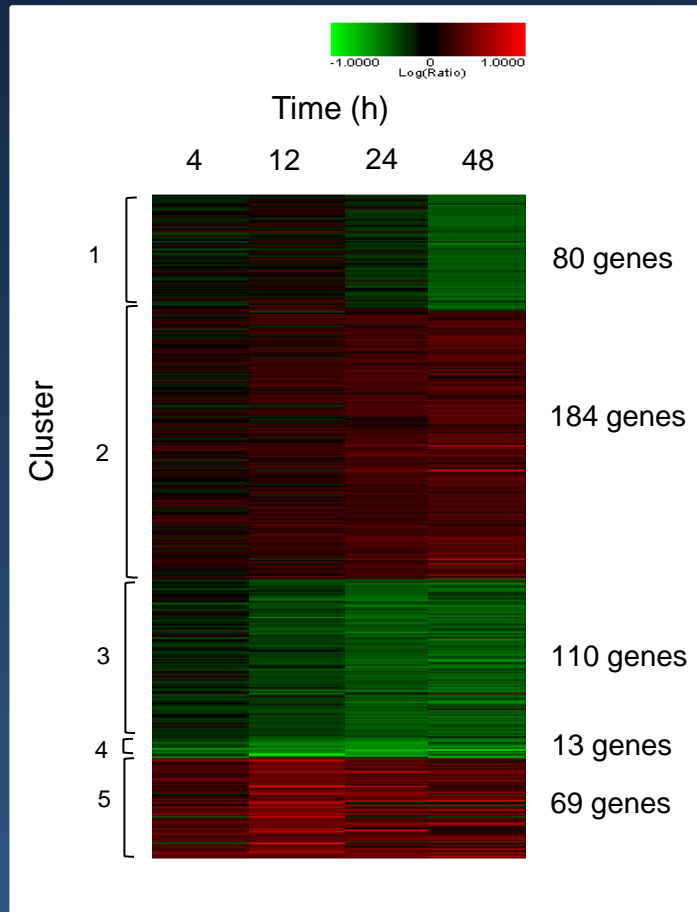
- ➔ Can we identify a genomic signature that reflects N or P limitation?
- ➔ How does the genome respond to nutrient addition?

Microarray analysis can identify signatures of N or P starvation in *K. brevis*



- Genes for N- or P- acquisition increased in expression in nutrient starved cells
- These genes were never before identified in dinoflagellates

Patterns of Gene Expression Following NO₃ Addition Reflect the Chloroplast Re-awakening and Increased Cellular Metabolism Within the First 4 Hours



Red – increased expression

Green - decreased expression

chloroplasts

nucleus



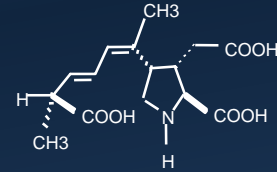
Increased Photosynthesis

Increased RNA biosynthesis

Decreased amino acid recycling

Increased Chloroplast – to – Nucleus signaling

Domoic Acid Posioning



1991

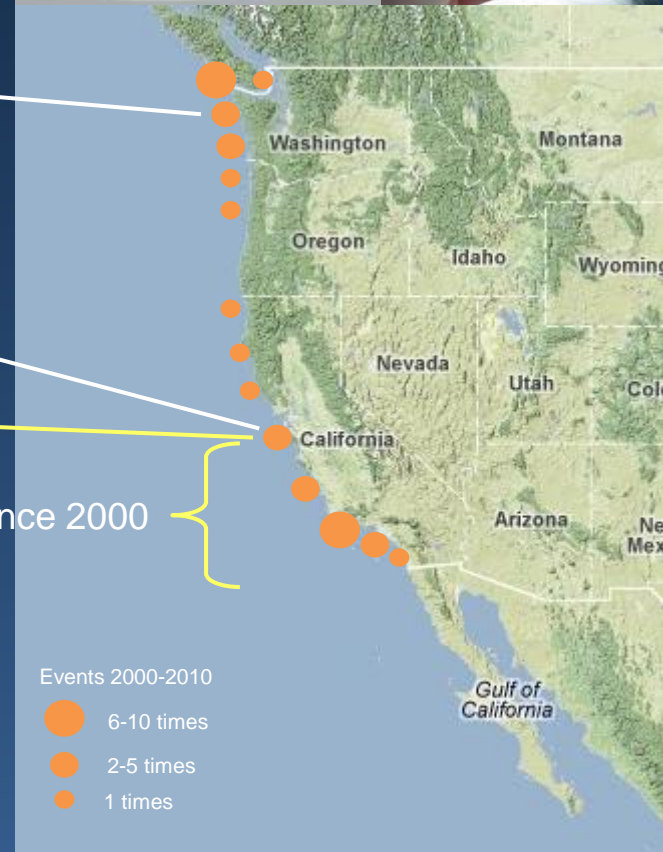
1991

1998

annually since 2000

Events 2000-2010

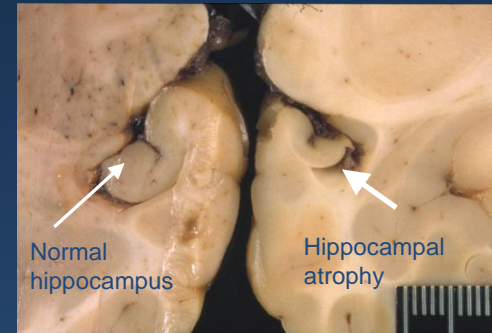
- 6-10 times
- 2-5 times
- 1 times



Domoic Acid Toxicity in California Sea Lions

Acute Exposure: strandings coincide with spring *Pseudo-nitzschia* blooms

- 1000s of sea lions stranded (mostly adult female)
- disorientation, scratching, ataxia, seizure
- lesions in brain and heart
- abortion, premature births, abnormal pups

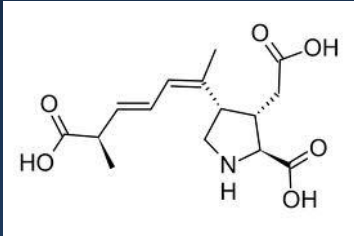


Chronic Exposure: epilepsy-like symptoms

- repeated, unpredictable seizure activity
- tagged, released animals show aberrant behavior
- young males show unusual aggression
- may reflect in utero exposure
- may reflect low dose, repeat exposure



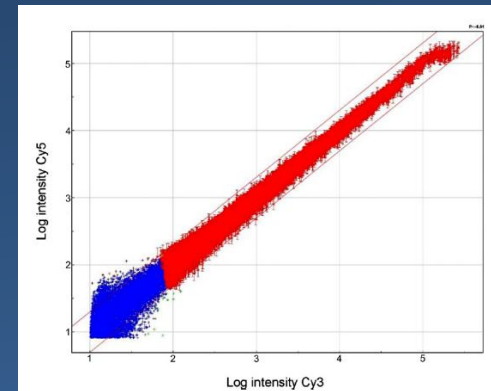
Microarray Profiling of Blood to Assess Exposure and Disease States in California Sea Lions



Domoic acid

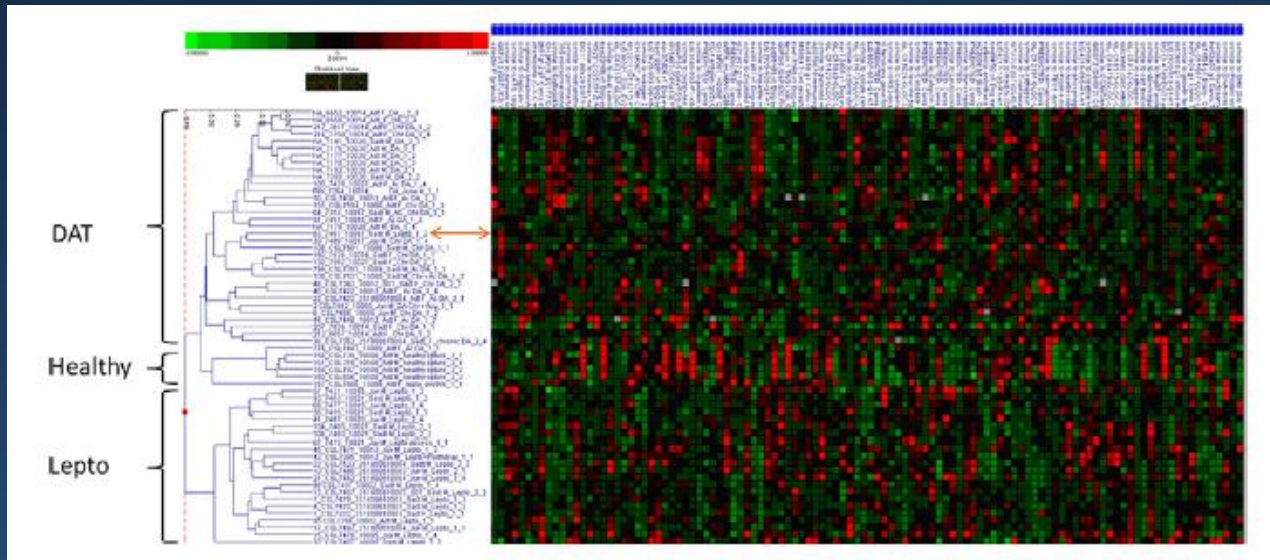


Leptospira



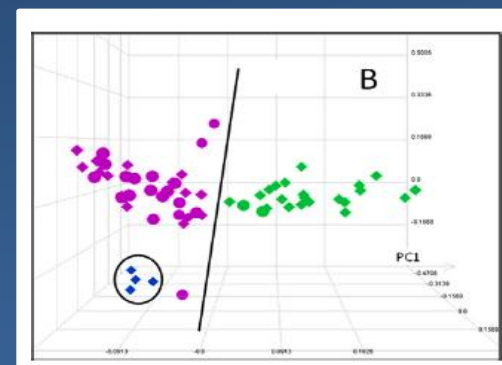
sea lion blood hybridized to dog array

- Blood RNA from sea lions of known health status was supplied by the Marine Mammal Center and analyzed by microarray
- Among 58 animals, hierarchical cluster analysis correctly grouped the domoic acid and leptospirosis animals based on the expression levels of 121 genes



- Principle components analysis clearly distinguishes the two disease states and healthy controls based on blood gene expression patterns.

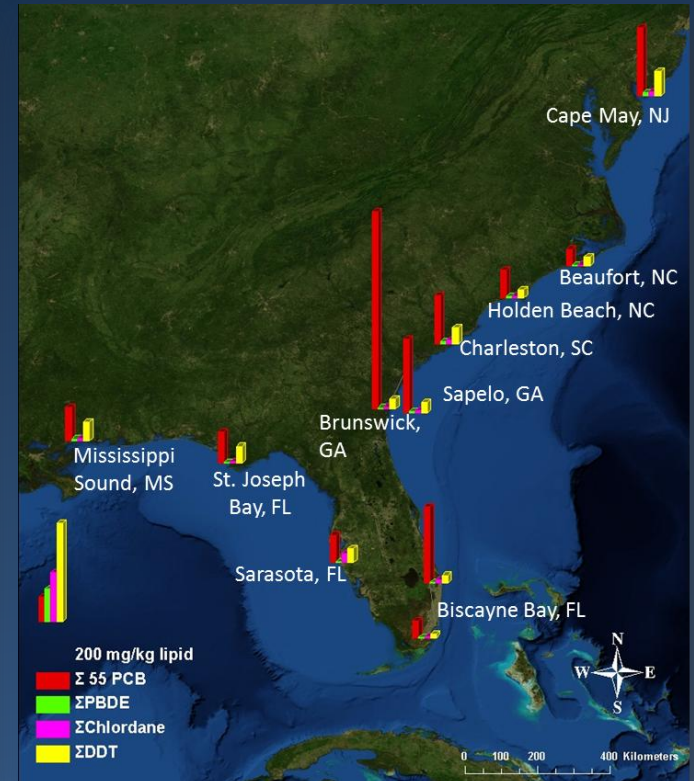
Purple - domoic acid
Green - leptospirosis



Bottlenose dolphins: sentinels for human health and environmental condition



Dolphin health assessment study sites.
Collaboration between NMFS OPR, NOS/NCCOS,
NIST, academic and state partners.

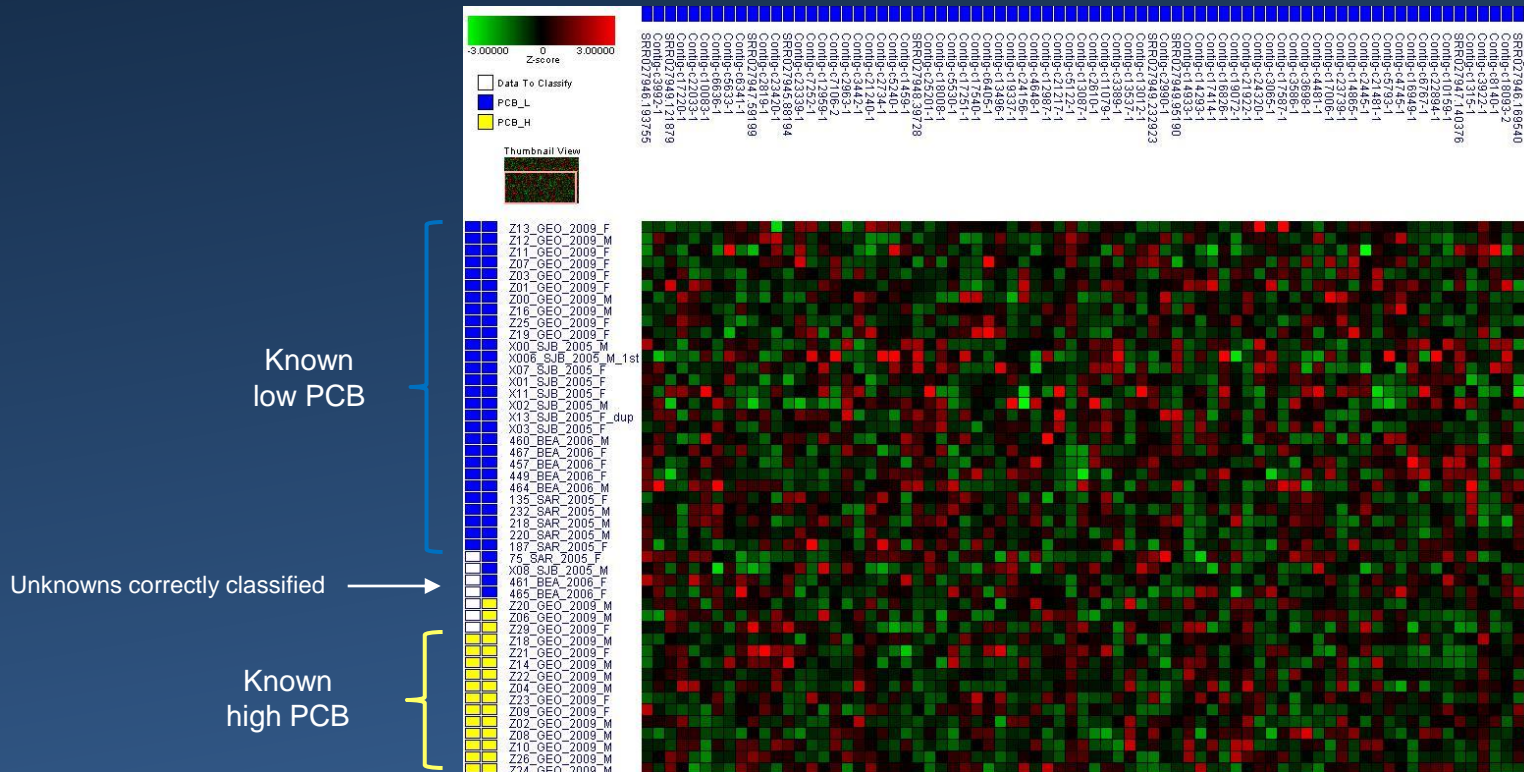


Levels of persistent organic
contaminants in dolphins. High
PCB levels in Georgia reflect
nearby superfund site.

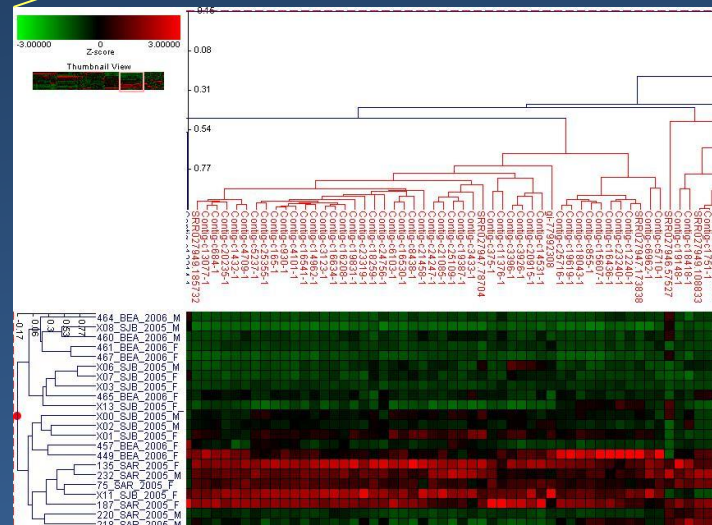
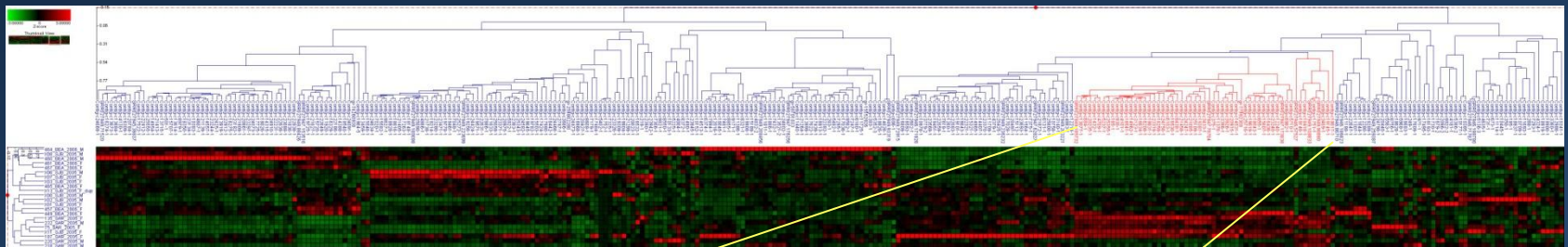
Dolphin microarray successfully classified animals with high PCBs using blood gene expression



K-nearest neighbor classifier algorithm



Dolphin microarray may be reporting a unique profile for animals in Sarasota Bay exposed to persistent red tide 2005-2006



Sarasota Bay 2005

HAB Genomics Lab (CCEHBR) and Marine Genomics Core Facility (HML) Staff and Students

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